suPAR-GUIDED ANAKINRA TREATMENT FOR VALIDATION OF THE RISK AND EARLY MANAGEMENT OF SEVERE RESPIRATORY FAILURE BY COVID-19: THE SAVE-MORE DOUBLE-BLIND, RANDOMIZED, PHASE III CONFIRMATORY TRIAL

STUDY PROTOCOL

Author: Evangelos J. Giamarellos-Bourboulis, MD, PhD

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Study Sponsor:

Hellenic Institute for the Study of Sepsis

88 Michalakopoulou Str., 115 25 Athens

e-mail: insepsis@otenet.gr

tel: +30 210 74 80 662

E. J. Giamarellos-Bourboulis 21.12.2020

DISCLOSURE OF PRINCIPAL INVESTIGATOR

Protocol Study Title: suPAR-GUIDED ANAKINRA TREATMENT FOR VALIDATION OF THE RISK AND EARLY MANAGEMENT OF SEVERE RESPIRATORY FAILURE BY COVID-19: THE SAVE-MORE DOUBLE-BLIND, RANDOMIZED, PHASE III CONFIRMATORY TRIAL

The herein protocol became known to myself by the Study Sponsor. I understand that the protocol remains as yet unpublished; I certify that all disclosed information to myself for this protocol will remain strictly confidential.

The Principal Investigator,

Print Name

Signature Date

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LIST OF ABBREVIATIONS

AE: adverse event

ALT: alanine aminotransferase

AST: aspartate aminotransferase

aPTT: activated partial thromboplastin time

BMI: body mass index

CI: confidence interval

COVID-19: Coronavirus 2019 disease

CRA: Clinical research associate

CRP: C-reactive protein

Ct: cycle

DMC: data monitoring committee

ECMO: extra corporeal membrane oxygenation

eCRF: electronic case report form

ED: emergency department

EDTA: ethylene-diamene-tetracetic acid

ETF-EMA: emergency task force of the European Medicines Agency for COVID-19

FiO_2: fraction of inspired oxygen

γGT: gamma-glutamyl transpeptidase

HR: hazard ratio

ICU: intensive care unit

IL: interleukin

INR: international normalized ratio

ISIC: International Study on Inflammation in COVID-19

ITT: intention-to-treat

IV: intravenous

LOCF: last observation carried forward

LRTI: lower respiratory tract infection

MCH: mean corpuscular hemoglobin

MCHC: mean corpuscular hemoglobin concentration

MCV: mean corpuscular volume

MV: mechanical ventilation

NaCl: sodium chloride

NIH: National Institute for Health

NIV: non-invasive mechanical ventilation

NPV: negative predictive value

PaO_2: arterial partial oxygen pressure

PP: per protocol

RCT: randomized clinical trial

RT-PCR: real-time polymerase chain reaction

SAE: serious adverse event

SAP: serum alkaline phosphatase

SARS-CoV-2: severe acute respiratory syndrome coronavirus-2

SIV: site initiation visit

SOC: standard-of-care

SpO_2: oxygen saturation

SRF: severe respiratory failure

STEAE: severe treatment-emergent adverse event

suPAR: soluble urokinase plasminogen activator receptor

TEAE: treatment-emergent adverse event

WHO: World Health Organization

WHO-CPS: WHO clinical progression scale

TRIAL SYNOPSIS

Background and Aim	The open-label interventional SAVE trial introduced the need to elaborate a novel strategy for the management of COVID-19: use one biomarker to predict the admitted patient who will develop SRF; and when tested positive start early anti-inflammatory treatment with anakinra to prevent progression into SRF. Plasma concentrations of suPAR ≥6 ng/ml are suggested as one suitable prognostic tool; its prognostic performance has been validated in prospective data collected by the ISIC consortium. In the interim analysis of the SAVE trial, the incidence of SRF among the 130 patients starting early anakinra treatment was 22.3% compared to 59.2% among parallel SOC comparators; 30-day mortality was 11.5% and 22.3% respectively. The SAVE-MORE is a pivotal, confirmatory, phase III randomized clinical trial (RCT) aiming to evaluate the efficacy and safety of early start of anakinra guided by suPAR in patients with LRTI by SARS-CoV-2 in improving the clinical state of COVID-19 over 28 days as measured by the ordinal scale of the 11-point WHO clinical progression scale (CPS).
Study design	Prospective multicenter, double-blind, randomized phase III clinical trial
Inclusion criteria	1. Age equal to or above 18 years 2. Male or female gender 3. In case of women, unwillingness to remain pregnant during the study period. 4. Written informed consent provided by the patient. For subjects without decision-making capacity, informed consent must be obtained from a legally designated representative following the national legislation in the Member State where the trial is planned. 5. Confirmed infection by SARS-CoV-2 virus 6. Findings in chest-X-ray or in chest computed tomography compatible with lower respiratory tract infection 7. Need for hospitalization for COVID-19. The need for hospitalization is defined by the attending physician taking into consideration clinical presentation, requirement for supportive care, potential risk factors for severe disease, and conditions at home, including the presence of vulnerable persons in the household. 8. Plasma suPAR ≥6ng/ml

Exclusion criteria	• Age below 18 years • Denial for written informed consent • Any stage IV malignancy • Any do not resuscitate decision • Any pO_2/FiO_2 (partial oxygen pressure to fraction of inspired oxygen) ratio less than 150 mmHg irrespective if the patient is under mechanical ventilation (MV) / non-invasive ventilation (NIV) / extracorporeal membrane oxygenation (ECMO) or not • Patient under MV or NIV or ECMO • Any primary immunodeficiency • Less than 1,500 neutrophils/mm^3 • Plasma suPAR less than 6 ng/ml • Known hypersensitivity to anakinra • Oral or IV intake of corticosteroids at a daily dose equal or greater than 0.4 mg/kg prednisone for a period greater than the last 15 days. • Any anti-cytokine biological treatment the last one month • Severe hepatic failure defined as Child-Pugh stage of 3 • End-stage renal failure necessitating hemofiltration or peritoneal hemodialysis • Pregnancy or lactation. Women of child-bearing potential will be screened by a urine pregnancy test before inclusion in the study • Participation in any other interventional trial
Intervention	• Treatment Arm 1: patients receiving standard-of-care (SOC) and placebo. Placebo is injected subcutaneously once daily for 10 days • Treatment Arm 2: patients receiving SOC and anakinra. Anakinra is injected subcutaneously as 100 mg once daily for 10 days Stratification per site and per region will take into consideration three strata i.e. the need of oxygen support (that is also driving classification into moderate and severe illness) the administration of dexamethasone as standard-of-care (SOC) therapy and BMI (body mass index). The drug should be administered on the same time ± 2 hours every day. In case the patient is discharged alive home before the completion of 10 days of treatment, treatment will stop prematurely. It is explicitly stated that the minimum number of days of treatment is seven.

Standard-of-care treatment	*For patients NOT in need of oxygen support with moderate illness* • Regular monitoring of vital signs including pulse oximetry • Anticoagulant prophylaxis as follows: Patients who are receiving anticoagulant or antiplatelet therapies for other underlying conditions should continue these medications. For the other patients pharmacological prophylaxis, such as low molecular weight heparin should be used according to local standards, to prevent venous thromboembolism, when not contraindicated. • Remdesivir treatment is reserved at the discretion of the treating physicians *For patients in need of oxygen support with severe illness* • Immediate implementation of oxygen support • Application of positioning and airway clearance management, as needed per the discretion of the treating physicians • Regular monitoring of vital signs including pulse oximetry • Regular monitoring for signs or symptoms suggestive of venous or arterial thromboembolism, such as stroke, deep venous thrombosis, pulmonary embolism or acute coronary syndrome, and proceed according to hospital protocols for diagnosis (such as laboratory tests and/or imaging) and further management. • Cautious treatment with intravenous fluids • Dexamethasone 6 mg IV or orally for up to 10 days or until hospital discharge whichever comes first; • Anticoagulant prophylaxis as follows: Patients who are receiving anticoagulant or antiplatelet therapies for other underlying conditions should continue these medications. For the other patients pharmacological prophylaxis, such as low molecular weight heparin should be used according to local standards, to prevent venous thromboembolism, when not contraindicated. • Remdesivir treatment is reserved at the discretion of the treating physicians Other concomitant drugs are allowed with the exception of any biological anti-

	cytokine drug targeting cytokines such as TNFα, IL-1 and IL-6.
Primary study outcome	The primary study outcome is the comparative 5-scale patient state evaluated from the 11-point WHO Clinical Progression ordinal Scale (CPS) between the two arms of treatment by Day 28. This will be expressed as the distribution of the frequencies of each score of the scale in each arm of treatment by Day 28.
Secondary study outcomes	The comparison of the following between the two arms of treatment: • Change of the measure of the 11-point of WHO Clinical Progression ordinal Scale (CPS) by Day 28 from baseline Day 1 (both absolute and relative changes) • Change of the measure of the 11-point of WHO Clinical Progression ordinal Scale (CPS) by Day 14 from baseline Day 1 (both absolute and relative changes) • Change of the SOFA score by Day 14 from baseline Day 1 (both absolute and relative changes) • Change of the SOFA score by Day 7 from baseline Day 1 (both absolute and relative changes) • Time until discharge from hospital • Time until discharge from the intensive care unit (this applies only for patients who will be admitted in the ICU) • Long-term safety by Day 60 • Long-term safety by Day 90 • Relative changes of circulating concentrations of suPAR, CRP, D-dimers, ferritin, and IL-6 by Day 7 from baseline Day 1 • Relative changes of circulating concentrations of suPAR, CRP, D-dimers, ferritin, and IL-6 by Day 4 from baseline Day 1 • Change of the viral load by Day 7 from baseline Day 1 (both absolute and relative changes) • Change of the viral load by Day 4 from baseline Day 1 (both absolute and relative changes) • Transcriptomic analysis that will also allow for lymphocyte cell subset analysis • Proteomic analyses • Relation of endpoints to duration of disease (from first symptoms) and timing of

	treatment initiation
Power of the study	The needed number of patients is based on the following hypothesis: • The distribution of the frequencies of the main categories of the WHO CPS of SOC comparators of the SAVE trial by Day 28 was: 21% death; hospitalized with severe disease 21%; hospitalized with moderate disease 13.7% and ambulatory mild disease 44.3% • The distribution of the frequencies of the main categories of the WHO CPS of anakinra-treated patients of the SAVE trial by Day 28 was: 10.9% death; hospitalized with severe disease 5.4%; hospitalized with moderate disease 6.2%; and ambulatory mild disease 77.5% • 90% power at the 5% level of significance are used • 1:2 randomization applies (one patient allocated to treatment Arm 1; two patients allocated to treatment Arm 2) Final calculation after adjusting for the design effect (DEFF): 600 patients need to be enrolled in total (200 patients in Arm 1; and 400 patients in Arm 2)

BACKGROUND

Generated evidence by the SAVE trial

Since March 2020 when the COVID-19 pandemic started in Europe, the Hellenic Institute for the Study of Sepsis has launched in Greece the SAVE clinical trial (suPAR-guided Anakinra treatment for Validation of the risk and Early management of severe respiratory failure by COVID-19) (EudraCT number 2020-001466-11; approval 38/20 of the National Ethics Committee of Greece, approval IS 028/20 of the National Organization for Medicine of Greece, ClinicalTrials.gov identifier, NCT04357366). The concept of the SAVE trial was that early recognition of the risk for the progression of patients with lower respiratory tract infection (LRTI) by the new coronavirus SARS-CoV-2 into severe respiratory failure (SRF) may guide anakinra therapy to prevent SRF. The tool that was used for the diagnosis of risk for SRF is the biomarker suPAR (soluble urokinase plasminogen activator receptor) at measurable concentrations in the blood ≥6 ng/ml. The trial was designed to be open-label non-randomized and the idea was το the start of treatment well before any sign of respiratory failure emerges. Patients hospitalized at tertiary hospitals during the same time period as the SAVE trial was ongoing and who were receiving the same standard-of-care (SOC) treatment were studied as comparators. An interim analysis was submitted to the National Organization for Medicines (number 108002/23.10/2020)[1]. In this interim analysis, 130 patients receiving anakinra treatment and SOC were analysed and they were compared to 130 patients receiving SOC. The 130 SOC parallel comparators were selected by propensity score matching to be fully matched to the anakinra-treated patients for age, comorbidities, severity scores on the day of hospital admission (i.e. APACHE II score, PSI, SOFA and WHO severity) and for the intake of azithromycin, hydroxychloroquine and dexamethasone. SRF was defined as any respiratory ratio (pO_2/FiO_2) less than 150 mmHg necessitating mechanical ventilation or non-invasive ventilation (NIV). The results of this analysis may be summarized as follows:

- The incidence of SRF was significantly decreased from 59.2% in the parallel standard-of-care (SOC) comparators (n= 130) to 22.3% among the 130 anakinra-treated patients (hazard ratio, 0.30; 95% confidence intervals 0.20-0.46; P: 4.6 x 10^{-8}).

- 30-day mortality was decreased from 22.3% in the SOC comparators to 11.5% among anakinra-treated patients (hazard ratio 0.49; 95% confidence intervals 0.25-0.97%; P: 0.041).
- Duration of stay at the intensive care unit was shortened with anakinra treatment compared to the SOC comparators for the patients who eventually developed SRF
- The median cost of hospitalization was significantly reduced from €2.398,40 among SOC comparators to €1.291,40 among anakinra-treated patients
- No safety concerns were raised.

The biomarker suPAR

Soluble urokinase plasminogen activator receptor (suPAR) is the soluble counterpart of the uPAR receptor which is anchored in the cell membrane of neutrophils and endothelial cells and which is cleaved after activation of the kalikrein system. suPAR can be quickly measured using the CE-IVD approved suPARnostic® assays (ViroGates, Denmark) using lateral flow quick tests or turbidimetric assays (on Roche, Siemens or Abbott platforms) and is therefore suitable for use as screening tool at the emergency department (ED). Concentrations less than 4 ng/ml have considerable negative predictive value (NPV) for death and suggest that the patient may be safety discharged from the ED. Concentrations more than 6 ng/ml advise in favour of hospital admission[2].

With the arrival of the COVID-19 pandemic, one small-scale study was conducted in 57 Greek patients. In this study[3], patients with LRTI by SARS-CoV-2 were divided into those with admission levels of suPAR ≥6 ng/ml and into those with admission suPAR less than 6 ng/ml. The incidence of SRF was 85.7% and 8.3% respectively. Multivariate Cox-regression analysis revealed admission suPAR ≥6 ng/ml to be the only independent predictor of the incidence of SRF even as early as 14 days ahead; gender, comorbidities, the absolute neutrophil count and C-reactive protein (CRP) were included in the analysis. This prognostic ability of suPAR was validated in two more publications: one in 369 patients[4] and another in 881 patients[5] who are participants of the ISIC (International Study of Inflammation in COVID-19) from the University of Michigan. Patients were divided into tertiles according to the admission suPAR levels at the ED i.e. less than 4.6 ng/ml, 4.6-6.86 ng/ml, and more

than 6.86 ng/ml. The incidence of SRF in patients in the highest suPAR tertile was 44.9% whereas it was 17.9% in the second tertile and 2.6% in the lowest tertile ($p<0.001$)[4].

As a consequence, there are cohorts of patients from several locations (USA, Greece, Denmark and Germany) supporting the use of suPAR levels ≥6 ng/ml as the biomarker to indicate early the patients at high likelihood for development of SRF:

- One original publication on 57 patients from Greece[3]
- One validation publication on 369 patients from the ISIC study[4]
- Validation un-published data on 881 patients from the ISIC study[5]
- Validation on the SOC parallel comparators of the SAVE study[1]

The above evidence strongly suggests that suPAR ≥6 ng/ml should be used as the selection tool for the early identification of patients at risk for SRF in the SAVE-MORE trial.

Anakinra

Anakinra is the recombinant soluble antagonist of interleukin (IL)-1 receptor. This drug has strong anti-inflammatory potential by inhibiting both IL-1α and IL-1β. The idea for the use of anakinra to prevent the development of SRF is coming from evidence suggesting that a) IL-1α is released from bronchial epithelial cells at the initial stages of LRTIs by viruses[6]; and b) hyper-production of IL-1β by circulating monocytes of patients at SRF from COVID-19[7]. The mode of action of anakinra makes it a relatively safe drug since it acts through scavenger excess IL-1α and IL-1β and not by inhibiting basal production so as to introduce risk of infection for the patients. According to the SmPC, the adverse events of anakinra are classified as very common (≥ 1/10), common (≥ 1/100 to < 1/10) and uncommon (≥ 1/1,000 to < 1/100) and they are presented in the Table 1[8].

Table 1 Adverse events of anakinra according to the SmPC

MedDRA Organ System	**Frequency**	**Undesirable Effect**
Infections and infestations	Common	Serious infections
Blood and lymphatic system disorders	Common	Neutropenia Thrombocytopenia
Immune system disorders	Uncommon	Allergic reactions including anaphylactic reactions, angioedema, urticaria and pruritus

Nervous system disorders	Very common	Headache
Hepatobiliary disorders	Uncommon	Hepatic enzyme increased
	Not known	Non-infectious hepatitis
Skin and subcutaneous tissue disorders	Very common	Injection site reaction
	Uncommon	Rash
Laboratory	Very common	Blood cholesterol increased

The list of severe adverse events (SAEs) and non-SAEs of the interim analysis of the SAVE trial proved that anakinra was safe among patients with COVID-19 (Table 2).

In the SAVE trial, the studied dose of anakinra was 100mg once daily and it was administered subcutaneously for 10 days. This daily dose is similar to the dose described at the SmPC of anakinra (Kineret®). The SAVE study can be considered as a) one dose-defining study; and b) confirmatory study of the safety of anakinra for COVID-19. Taking into consideration the below evidence:

- The dose of anakinra in the SAVE study is similar to the daily dose of the SmPC of anakinra
- This dose is already known to have a positive benefit: risk ratio as per SmPC in the approved indications
- Anakinra administered subcutaneously 100mg once daily for 10 days in COVID-19 in SAVE study was proven to be safe with no new safety signals

This dose is chosen for the SAVE-MORE trial

Provision for the placebo treatment group

The use of placebo in the SAVE-MORE trial is generating concerns to reduce the possible harm that may be caused in these patients taking into consideration the results of the SAVE study. To limit all risk for the placebo group the following are provisioned:

- The randomization to placebo and active treatment will be 1:2
- All participants in the SAVE-MORE trial will receive specific SOC
- The incidence of the primary study endpoint will be closely monitored by a DMC board and the trial will be prematurely stopped, if needed

PROTOCOL ADVICE

The SAVE-MORE pivotal, confirmatory, phase III randomized clinical trial (RCT) was designed based on advice that was received from the Emergency Task Force (ETF) of the European Medicines Agency (EMA) for COVID-19 (document EMA/659928/2020) of the Clinical Studies and Manufacturing Task Force (TCS) of the EMA; dated December 3 2020) on Version 1.0 of the trial protocol (provided in Appendix II)

AIM OF THE STUDY

The SAVE-MORE is a pivotal, confirmatory, phase III randomized clinical trial (RCT) aiming to evaluate the efficacy and safety of early start of anakinra guided by suPAR in patients with LRTI by SARS-CoV-2 in improving the clinical state of COVID-19 over 28 days as measured by the ordinal scale of the 11-point WHO clinical progression scale (CPS).

STUDY DESIGN

This will be a prospective double-blind, randomized clinical trial that will take place in European study sites (Appendix I). The study protocol will be approved by the Institutional Review Boards (when required) and by the National Regulatory authorities. The study will be registered at Clinicaltrials.gov before enrolment of the first patient. The list of study sites in Greece is provided in Appendix I.

Study population

Patients who meet ALL the following inclusion criteria and who do not meet any of the following exclusion criteria are allowed to be enrolled:

Inclusion criteria

1. Age equal to or above 18 years
2. Male or female gender
3. In case of women, unwillingness to remain pregnant during the study period.
4. Written informed consent provided by the patient. For subjects without decision-making capacity, informed consent must be obtained from a legally designated representative following the national legislation in the Member State where the trial is planned.

5. Confirmed infection by SARS-CoV-2 virus
6. Findings in chest-X-ray or in chest computed tomography compatible with lower respiratory tract infection
7. Need for hospitalization for COVID-19. The need for hospitalization is defined by the attending physician taking into consideration clinical presentation, requirement for supportive care, potential risk factors for severe disease, and conditions at home, including the presence of vulnerable persons in the household.
8. Plasma suPAR ≥6ng/ml

Exclusion criteria

- Age below 18 years
- Denial for written informed consent
- Any stage IV malignancy
- Any do not resuscitate decision
- Any pO_2/FiO_2 (partial oxygen pressure to fraction of inspired oxygen) ratio less than 150 mmHg irrespective if the patient is under mechanical ventilation (MV) / non-invasive ventilation (NIV) / extracorporeal membrane oxygenation (ECMO) or not
- Patient under MV or NIV or ECMO
- Any primary immunodeficiency
- Less than 1,500 neutrophils/mm^3
- Plasma suPAR less than 6 ng/ml
- Known hypersensitivity to anakinra
- Oral or IV intake of corticosteroids at a daily dose equal or greater than 0.4 mg/kg prednisone for a period greater than the last 15 days.
- Any anti-cytokine biological treatment the last one month
- Severe hepatic failure defined as Child-Pugh stage of 3
- End-stage renal failure necessitating hemofiltration or peritoneal hemodialysis
- Pregnancy or lactation. Women of child-bearing potential will be screened by a urine pregnancy test before inclusion in the study
- Participation in any other interventional trial

Table 2 List of SAEs and non-SAEs captured until Day 14 and reported in the SAVE interim analysis

	Parallel Standard-of-care (N=130)	Standard-of-care + Anakinra (N=130)	*P*-Value
At least one SAE by day 14, No. (%)	63 (48.5)	32 (24.6)	1.0×10^{-4}
Extended hospitalization	63 (48.5)	32 (24.6)	1.0×10^{-4}
Death	16 (12.3)	6 (4.6)	0.043
Shock	56 (43.1)	27 (20.8)	1.6×10^{-5}
Acute kidney injury	37 (28.5)	15 (11.5)	0.001
Any bacterial infection	30 (23.1)	9 (6.9)	4.0×10^{-4}
Thromboembolic event	5 (3.8)	2 (1.5)	0.188
Pulmonary edema	0 (0)	1 (0)	1.00
At least one AE by day 14, No. (%)	89 (68.5)	85 (65.4)	0.693
Gastrointestinal disturbances	9 (6.9)	15 (11.5)	0.284
Electrolyte derrangements	41 (31.5)	35 (26.9)	0.496
Elevated liver function tests	51 (39.2)	40 (30.8)	0.193
Anemia	26 (20.0)	22 (16.9)	0.632
Leukopenia	3 (2.3)	11 (8.5)	0.051
Thrombopenia	7 (5.4)	9 (6.9)	0.797
Headache	2 (1.5)	4 (3.1)	0.684
Allergic reaction	7 (5.4)	4 (3.1)	0.540
Any heart arrhythmia	22 (16.9)	9 (6.9)	0.020

Screening for eligibility

No study related procedure will be performed prior obtaining written inform consent form. Screening is performed under the following steps:

- Step 1: The patient is screened for the exclusion criteria. If he meets any of them, he cannot be enrolled. If he does not meet any of them, he remains eligible and screening proceeds to step 2
- Step 2: The patient is screened for inclusion criteria 1 to 7. If he meets these criteria, he remains eligible and screening proceeds to step 3.
- Step 3: 22 ml of whole blood is drawn after venipuncture of one forearm vein under aseptic conditions; 3ml is collected into one EDTA-coated tube to be used for the measurement of suPAR and the remaining is aliquoted as described in the section Laboratory Techniques. A commercialized quick blood test with suPARnostic® Quick Triage (Virogates S/A, Blokken 45, 3460 Birkerød, Denmark) or suPARnostic® turbilatex will take place, to determinate in a very short time (20 min) suPAR levels in human EDTA-plasma. In the Quick Triage test, the sample (10 μl of plasma) will be incubated and handled, according to the manufacturer's instructions, attached to a provided reader and the results will be displayed through LF Software. The measurement is the result of a lateral flow immunoassay (LFIA) and constitutes a quantitative measurement (in ng/ml) of plasma suPAR levels, provided that suPAR values are detected within the range of 2-15 ng/ml, to be considered accurate. If suPAR is found ≥ 6 ng/ml, the patient can be enrolled in the study. In case screening fails or the time until start of the study drug exceeds 24 hours, the remaining collected amount of blood is destroyed.

Intervention

Patients will be randomly assigned 1:2 to treatment Arm 1 and treatment Arm 2. A separate randomization computer-generated chart will be applied in each study site. Stratification will be made based on participating sites or regions. Stratification per site and per region will take into consideration three strata i.e. the need of oxygen support (that is also driving classification into moderate and severe illness) the administration of dexamethasone as standard-of-care (SOC) therapy and BMI (body

mass index) (for more details see the Section Study Drug). The two groups of treatment will be as follows:

- Treatment Arm 1: patients receiving standard-of-care (SOC) and placebo. Placebo is injected subcutaneously once daily for 10 days
- Treatment Arm 2: patients receiving SOC and anakinra. Anakinra is injected subcutaneously as 100 mg once daily for 10 days

The drug should be administered on the same time ± 2 hours every day. In case the patient is discharged alive home before the completion of 10 days of treatment, treatment will stop prematurely. It is explicitly stated that the minimum number of days of treatment is seven.

See section of SOC treatment and other co-administered drugs.

Study drug

The placebo/active comparator will be prepared by one un-blinded pharmacist at each study site. The study team of each study site should have at least two un-blinded study pharmacists. Once one patient becomes eligible for study enrolment, the un-blinded pharmacist will be logged-in through one individual username and password into a safe and fully independent page of the study web-portal. There the un-blinded study pharmacist will fill the code of the patient, the severity of illness (moderate or severe), type of SOC (intake or no intake of dexamethasone) and the BMI (more than 30 or ≤30) and receive feedback information on allocation treatment arm.

In case the patient is allocated to Arm 1, the un-blinded pharmacist will fill one syringe with 0.67 ml sodium chloride (NaCl) 0.9%. One separate NaCl 0.9% ampule will be provided per patient of Arm 1. The syringe will be same in appearance to the syringe of Arm 2. Then the un-blinded pharmacist will cover the syringe with one corresponding label (see below) and deliver this to one blinded study pharmacist and one blinded study nurse for subcutaneous injection. The study team of each study site should have at least two blinded study pharmacists. At the end of the injection, the empty syringe is stored for accountability.

In case the patient is allocated to Arm 2, the un-blinded pharmacist will bring one pre-filled ready-to-use syringe at room temperature. Anakinra pre-filled syringes need to be stored at 2-8°C at the study site at a refrigerator with recording of

temperature. In case recording indicates deviation of temperature below 0°C or above 10°C for more than a day, stored syringes need to be replaced by the Sponsor. Then the un-blinded pharmacist will cover the syringe with one corresponding label (see below) and deliver this to one blinded study pharmacist and one blinded study nurse for subcutaneous injection. The study team of each study site should have at least two blinded study pharmacists. At the end of the injection, the empty syringe is stored for accountability.

On each label there will be one digit code composed of nine characters. The first two characters are the letters SM, from the initials of the study. The third and fourth characters are letters and denote the study site. The fifth to seventh characters denote the number of the patient at the specific study site. The eighth and ninth characters refer to the day of sampling. For example, the code SMAB00204 refers to study site AB, patient number 002 at that study site on treatment day 4.

Standard-of-care (SOC) treatment

Based on the disease severity of patients enrolled in the SAVE trial[1], it is anticipated that patients who will participate in the SAVE-MORE trial will have moderate or severe illness. According to the classification of severity published by the World Health Organization (WHO)[9], these are defined as follows for patients with LRTI:

- *Moderate illness*: clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) but no signs of signs of severe pneumonia, including saturation of oxygen (SpO2) ≥90% on room air.
- *Severe disease*: clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: respiratory rate >30 breaths/minute; severe respiratory distress; or SpO2 <90% on room

These two stages of the severity of the illness will be considered as independent strata of randomization (see also section of Intervention and study drug)

Current evidence suggests that the treatment algorithm for the management of COVID-19 for moderate and severe illness is regularly updated. It is anticipated that the overall duration of the study will be short (i.e. close to two months). To this end,

the SOC administered to patients enrolled in the SAVE-MORE study is not expected to be changed by the WHO until the end of the trial. Taking onto consideration: a) the current of algorithm for management of COVID-19 by the WHO[9]; b) the current algorithm for management of COVID-19 by the National Institute for Health (NIH)[10]; and c) the WHO suggestion for remdesevir[11], the SOC for patients enrolled in the SAVE-MORE study will contain the following:

For patients NOT in need of oxygen support with moderate illness

- Regular monitoring of vital signs including pulse oximetry
- Anticoagulant prophylaxis as follows:

Patients who are receiving anticoagulant or antiplatelet therapies for other underlying conditions should continue these medications. For the other patients pharmacological prophylaxis, such as low molecular weight heparin should be used according to local standards to prevent venous thromboembolism, when not contraindicated.

- Remdesivir treatment is reserved at the discretion of the treating physicians

For patients in need of oxygen support with severe illness

- Immediate implementation of oxygen support
- Application of positioning and airway clearance management, as needed per the discretion of the treating physicians
- Regular monitoring of vital signs including pulse oximetry
- Regular monitoring for signs or symptoms suggestive of venous or arterial thromboembolism, such as stroke, deep venous thrombosis, pulmonary embolism or acute coronary syndrome, and proceed according to hospital protocols for diagnosis (such as laboratory tests and/or imaging) and further management.
- Cautious treatment with intravenous fluids
- Dexamethasone 6 mg IV or orally for up to 10 days or until hospital discharge whichever comes first;
- Anticoagulant prophylaxis as follows:

Patients who are receiving anticoagulant or antiplatelet therapies for other underlying conditions should continue these medications. For the other patients pharmacological prophylaxis, such as low molecular weight heparin should be used according to local standards to prevent venous thromboembolism, when not contraindicated.

- Remdesivir treatment is reserved at the discretion of the treating physicians

Other concomitant drugs are allowed with the exception of any biological anti-cytokine drug targeting cytokines such as TNFα, IL-1 and IL-6.

The need of oxygen support (that is also driving classification into moderate and severe illness) and the administration of dexamethasone as SOC will be a independent strata of randomization (see also section of Intervention and study drug)

Patients' visits (Appendix III)

Day 1

This visit will take place on the morning of the day of the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-morbidities, SOFA (sequential organ failure assessment) score (see Appendix IV), co-administered drugs, past-history, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases and the percentage of lung infiltrates (if available, see Appendix V)
- Recording of the measure of the 11-point of WHO Clinical Progression ordinal Scale (CPS) (see section of primary endpoint and Appendix VI for definition).
- Collection of sample from the upper airway for RT-PCR testing for SARS-CoV-2
- Sampling of 19 ml of venous blood. This will be analyzed as described at the section Laboratory procedures. In case the time interval between blood sampling for screening and Day 1 is less than 24 hours, the blood collected during screening will be collected.
- Administration of the study drug (placebo/active comparator)
- Recording of the time interval between start of symptoms and initiation of the study drug (placebo/active comparator)

Day 2

This visit will take place on the morning of the second day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix V for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug (placebo/active comparator)

Day 3

This visit will take place on the morning of the third day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug (placebo/active comparator)

Day 4

This visit will take place on the morning of the fourth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Collection of sample from the upper airway for RT-PCR testing for SARS-CoV-2
- Sampling of 19 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

Day 5

This visit will take place on the morning of the fifth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug (placebo/active comparator)

Day 6

This visit will take place on the morning of the sixth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug (placebo/active comparator)

Day 7

This visit will take place on the morning of the seventh day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of SOFA score (see Appendix IV), co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

- Collection of sample from the upper airway for RT-PCR testing for SARS-CoV-2
- Sampling of 19 ml of venous blood. This will be analyzed as described at the section Laboratory procedures.
- Administration of the study drug (placebo/active comparator)

Day 8

This visit will take place on the morning of the eighth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug (placebo/active comparator)

The visit may take place by phone call or through the internet in case of hospital discharge. In that case the only captured information will be:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 9

This visit will take place on the morning of the ninth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug (placebo/active comparator)

The visit may take place by phone call or through the internet in case of hospital discharge. In that case the only captured information will be:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 10

This visit will take place on the morning of the tenth day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Administration of the study drug (placebo/active comparator)

The visit may take place by phone call or through the internet in case of hospital discharge. In that case the only captured information will be:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 14

This visit will take place on the morning of the 14^{th} day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of SOFA score (see Appendix IV), co-administered drugs, absolute blood cell count and differential (if available), biochemistry (if available), microbiology (if available), oxygen saturation and blood gases
- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

The visit may take place by phone call or through the internet in case of hospital discharge. In that case the only captured information will be:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 28

This visit will take place on the morning of the 28th day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Collection of sample from the upper airway for RT-PCR testing for SARS-CoV-2 (this is done only for asymptomatic patients)

The visit may take place by phone call or through the internet in case of hospital discharge. In that case the only captured information will be:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)
- Collection of sample from the upper airway for RT-PCR testing for SARS-CoV-2 (this is done only for asymptomatic patients). Sampling is done either with the patient visiting the study site for sample collection and testing or by the investigators visiting the patients at the place of residence for sample collection and testing

Day 60

This visit will take place on the morning of the 60th day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

The visit may take place by phone call or through the internet in case of hospital discharge. In that case the only captured information will be:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

Day 90

This visit will take place on the morning of the 60th day from the start of treatment with the study drug. The following procedures will be done on that day:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition).
- Recording of adverse events (AE) and severe adverse events (SAE)

The visit may take place by phone call or through the internet in case of hospital discharge. In that case the only captured information will be:

- Recording of the measure from the 11-point of WHO CPS (see section of primary endpoint and Appendix VI for definition)
- Recording of adverse events (AE) and severe adverse events (SAE)

LABORATORY PROCEDURES

Blood samples coming from day 1 (or screening visit in case they are drawn less than 24 hours apart), day 4 and day 7 will be collected as follows: a) 3 ml into one EDTA coated tubes for complete blood cell counting; b) 5ml into one sterile tube for complete biochemistry; c) 4 ml into one heparin-coated tube for coagulation tests; d) 4 ml into one citrate-coated tube for the isolation of plasma and protein biomarker analysis; and e) 3 ml into one PAXgene tube for transcriptomic analysis. In case screening fails, the collected amount of blood is destroyed.

Samples will be shipped from study sites into the study central lab that will be the Laboratory of Immunology of Infections at the 4th Department of Internal Medicine at ATTIKON University General Hospital or any other subcontracting lab collaborating with the Sponsor.

The following safety complete cell counting tests will be done:

- Total red blood cell count, MCV, MCH and MCHC
- Total white blood cell count and differential
- Total platelet cell count

The following safety biochemistry lab tests will be done:

- Glucose, urea, creatinine
- AST, ALT, γGT, SAP, total bilirubin and direct bilirubin
- Electrolytes (Na, K, Ca)
- Triglycerides
- C-reactive protein
- Ferritin
- Interleukin-6
- suPAR

The following safety coagulation lab tests will be done:

- INR, aPTT, fibrinogen
- D-dimers

Viral load will be expressed by the number of cycles (Ct) needed for the RT-PCR reaction for SARS-CoV-2 to be positive.

Isolated plasma will be subject to full Olink proteomic analysis and transcriptomic analysis at the Department of Internal Medicine of Radboud University at Nijmegen, The Netherlands. For the purpose of analysis, patients will be split into one discovery set cohort and into one validation set. The discovery-set will be composed from samples coming from 75 patients allocated to treatment Arm 1 of all three time points of follow-up (days 1, 4 and 7) equally coming from each randomization stratum; and from 75 patients allocated to treatment Arm 2 of all three time points of follow-up (days 1, 4 and 7) equally coming from each randomization stratum. The remaining 450 patients will be included in the validation set and they will be analysed only for the individual proteins and genes that will be derived from the discovery-set. The analysis of PAXgenes will also allow perform analysis on lymphocyte subsets.

STUDY OUTCOMES

Primary study outcome

The primary study outcome is the comparative 5-scale patient state evaluated from the 11-point WHO Clinical Progression ordinal Scale (CPS) between the two

arms of treatment by Day 28 (see Appendix IV). This will be expressed as the distribution of the frequencies of each score of the scale in each arm of treatment by Day 28.

Secondary study outcomes

The comparison of the following between the two arms of treatment will be the study secondary outcomes:

- Change of the measure of the 11-point of WHO Clinical Progression ordinal Scale (CPS) by Day 28 from baseline Day 1 (both absolute and relative changes)
- Change of the measure of the 11-point of WHO Clinical Progression ordinal Scale (CPS) by Day 14 from baseline Day 1 (both absolute and relative changes)
- Change of the SOFA score by Day 14 from baseline Day 1 (both absolute and relative changes)
- Change of the SOFA score by Day 7 from baseline Day 1 (both absolute and relative changes)
- Time until discharge from hospital
- Time until discharge from the intensive care unit (this applies only for patients who will be admitted in the ICU)
- Long-term safety by Day 60
- Long-term safety by Day 90
- Relative changes of circulating concentrations of suPAR, CRP, D-dimers, ferritin, and IL-6 by Day 7 from baseline Day 1
- Relative changes of circulating concentrations of suPAR, CRP, D-dimers, ferritin, and IL-6 by Day 4 from baseline Day 1
- Change of the viral load by Day 7 from baseline Day 1 (both absolute and relative changes)
- Change of the viral load by Day 4 from baseline Day 1 (both absolute and relative changes)
- Transcriptomic analysis that will also allow for lymphocyte cell subset analysis
- Proteomic analyses
- Relation of endpoints to duration of disease (from first symptoms) and timing of treatment initiation

Exploratory study outcomes

The comparison of the following between the two arms of treatment will be the study exploratory outcomes:

- The cost of hospitalization
- The 11-point of WHO Clinical Progression ordinal Scale (CPS) by Day 60; this will be expressed as the distribution of the frequencies of each score of the scale in each arm of treatment by Day 60.
- The 11-point of WHO Clinical Progression ordinal Scale (CPS) by Day 90; this will be expressed as the distribution of the frequencies of each score of the scale in each arm of treatment by Day 90.
- The over-time curve of the measures of the 11-point of WHO Clinical Progression ordinal Scale (CPS) between Days 1 and 14.

NUMBER OF PATIENTS

The needed number of patients is based on the following hypothesis:

- The distribution of the frequencies of the main categories of the WHO CPS of SOC comparators of the SAVE trial by Day 28 was: 21% death; hospitalized with severe disease 21%; hospitalized with moderate disease 13.7% and ambulatory mild disease 44.3%
- The distribution of the frequencies of the main categories of the WHO CPS of anakinra-treated patients of the SAVE trial by Day 28 was: 10.9% death; hospitalized with severe disease 5.4%; hospitalized with moderate disease 6.2%; and ambulatory mild disease 77.5%
- 90% power at the 5% level of significance are used
- 1:2 randomization applies (one patient allocated to treatment Arm 1; two patients allocated to treatment Arm 2)

Final calculation after adjusting for the design effect (DEFF): 600 patients need to be enrolled in total (200 patients in Arm 1; and 400 patients in Arm 2).

STATISTICAL ANALYSIS

All baseline quantitative characteristics between the two groups of treatment will be compared by the Student's t-test for parametric variables and by the Mann-Whitney U test for non-parametric variables.

The analysis of the primary study outcome (i.e. WHO CPS by Day 28) will be done according to the ITT principle including the Intent-to-Treat (ITT) population[13]. Imputation of missing data will be done by the principle of Last Observation Carried Forward (LOCF). In this analysis the last measured value of the endpoint is imputed to all subsequent, scheduled, but missing, evaluations[14].

Since the primary endpoint is 5-scale ordinal variable, the homogeneity of the frequency distributions across the two groups of treatment will be tested with the chi-square for trends test, to test if there will be a shift of the frequency distribution between the two groups. This will be followed by subgroup analyses using the same statistical procedure with the following pre-specified subgroups that will also be used for stratification in the randomization process:

- Severity of illness per WHO classification[9]
- Intake of dexamethasone in the standard-of-care
- Body mass index (BMI; $\leq$30 or >30)

Comparisons between groups will be validated by analysis of covariance following the EMA Guideline on adjustment for baseline covariates in clinical trials[13]. Another validation for adjustment of covariates will also be performed: After the primary outcome will be transformed to a binary variable with values less than 6 and values equal to or greater than 6, it will be subjected to multivariate logistic regression with group and the above three covariates as independent predictors. For subgroups of interest the Cochrane-Mantel-Haenszel test will also be performed. Finally, comparisons of the primary endpoint between the two Arms of treatment will be done by the Mann-Whitney non-parametric test.

Five sensitivity analyses will be done between the two Arms of treatment:

- Analysis including all patients receiving study drug for at least seven days
- Comparison of the treatment-effect provided by the unadjusted comparison and the adjusted model[13]

- Complete case analysis (i.e. ignoring incomplete data) and comparison to the full set analysis[14]
- Responder analysis treating all missing values as failures[14]
- Analysis in the PP population; patients receiving at least one dose of the study drug are the per protocol (PP) population.

Any p-value below 0.05 will be considered significant.

DATA MONITORING COMMITTEE (DMC)

The following have already been contacted and agreed to participate in the DMC:

- Michael Niederman, Cornell University, New York, USA, as Chairman
- Konrad Reinhart, Charité University, Berlin, Germany, as Member
- Jos WM van der Meer, Radboud University, Nijmegen, The Netherlands, as Member

Meetings will take place before the start of the trial and at pre-defined time points corresponding to 40%, 70% and 100% of the enrolment. Meetings will run by safe electronic access to the website of the HISS. During the meetings at 40% and 70% of enrolment, DMC will be informed in a blinded way the difference between the two Arms of Treatment for the primary endpoint and for serious treatment-emergent adverse events. At the end of each meeting, all members will sign the minutes and a statement whether allowing the study to continue as per agreed criteria. The DMC will finally meet after the final analysis is available.

ADVERSE EVENTS

Treatment-emergent adverse events (TEAEs) and Serious TEAEs (STEAEs) along with AEs/SAEs where applicable will be captured from baseline until the last patient's evaluation. Investigators should monitor subjects for TEAEs and are responsible for recording ALL adverse events and adverse reactions occurring to a patient during the trial. A TEAE is any undesirable and unintended medical occurrence in a subject administered a pharmaceutical product and which does not

necessarily have a causal relationship with this treatment. The STEAE may be a sign, a symptom or an abnormal laboratory finding.

Following advice from the ETF of the EMA, long-term TEAE will be captured until day 90. Events comprised in the primary study outcome (i.e. the 11-point WHO CPS) will not be reported as TEAE[14]. These events are: need for medical treatment with NIV; need for medical treatment with MV; need for medical treatment with dialysis or ECMO; and death.

If a TEAE meets any of the following criteria, it is considered severe:

- **Life-threatening situation** The subject was at risk of death at the time of the adverse event/experience. It does not refer to the hypothetical risk of death if the adverse event/adverse reaction were more severe or were to progress.
- **Inpatient hospitalization** or prolongation of existing hospitalization.
- **Persistent or significant disability/incapacity** Any TEAE having an outcome that is associated with a substantial disruption of the ability to carry out normal life functions, including the ability to work. This is not intended to include transient interruption of daily activities.
- **Congenital anomaly/birth defects** Any structural abnormality in subject's offspring that occurs after intrauterine exposure to treatment.
- **Important medical events/experiences** that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event/experience when, based upon appropriate medical judgment, **they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above,** i.e., death, a life-threatening adverse event/experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Examples of such medical events/experiences include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
- **Spontaneous and elective abortions** experienced by study subject.

Grading of severity

The severity of the TEAE shall be graded as:

- **Mild** the TEAE is transient and well tolerated by the patient

- **Moderate** the TEAE causes discomfort and affects the usual activities of the patient.
- **Severe** the TEAE affects the usual activities of the patient to an important degree and may cause disability or be life-threatening.

Relationship to the drug

The investigator will use the following definitions to assess the relationship of the adverse event to study drug:

- **Probably Related**: The TEAE has a strong time relationship to the drug or relapses if re-induced, and another etiology is improbable or clearly less probable.
- **Possibly Related**: The TEAE has a strong time relationship to the drug and an alternative aetiology is as probable or less probable.
- **Probably not Related**: The TEAE has a slight or no time relationship to the drug and/or there is a more probable alternative aetiology.
- **Unrelated**: The TEAE is due to an underlying or concomitant disease or to another pharmaceutical product and is not related to the drug (no time relationship and a much more probable alternative aetiology).

If an investigator's opinion of possibly related, probably not related or not related to study drug is given, an alternate etiology must be provided by the investigator. Please note that a severe TEAE is not necessarily serious, as the term severe is a measure of intensity. Individual un-blinding thought to be necessary for the management of an adverse event will be documented in the subject Case Report Form. All Investigators must report every STEA and evaluate the severity and possible causality with the study drug according to aforementioned criteria. All TEAEs are reported to Sponsor. The sponsor is responsible for evaluation of all TEAEs. All STEAEs/SAEs must be reported within 24 hours by completion of the TEAE and faxed to the Hellenic Institute for the Study of Sepsis (HISS). The Sponsor must evaluate whether a TEAE is expected or not. A STEAE/SAE may qualify for expedited reporting to regulatory authorities if it is determined to be a suspected, unexpected serious adverse reaction (SUSAR). The Sponsor is responsible for submitting expedited safety reports to the appropriate regulatory agency for all confirmed SUSARs. In the case of a fatal or life-threatening SUSAR, the Sponsor will notify the appropriate regulatory agency as soon as possible but in no case later than 7 calendar days after The Sponsor's initial receipt of the information. For a non-life-

threatening SUSAR, the report will be submitted no later than 15 days after the Sponsor is made aware of the event. The Sponsor has the obligation to submit annually a drug safety updated report (DSUR) according to global experience to appropriate regulatory authorities. The electronic submission to Eudravigilance will be performed through the Organisation ID: HISS.

CLINICAL DATA ENTRY

Clinical data entry will be done by the investigators of each study site into the eCRF platform of the safe website of HISS. The PI and sub-Is of each study site will be provided separate usernames and passwords that will allow them access to the ePortal at www.sepsis.gr.

STUDY MONITORING PLAN

Clinical research associate (CRAs) of the Sponsor will establish and maintain regular contact between the investigator and the sponsor. Each CRA will be provided separate usernames and passwords that will allow them access to the ePortal at www.sepsis.gr.CRAs will evaluate the competence of each study center, informing the sponsor about any problems relating to facilities, technical equipment or medical staff. During the study, CRAs will check that written informed consent has been obtained from all subjects correctly according to principles of Declaration to Helsinki, GCP, and applicable regulatory requirements and that data are recorded correctly and completely. CRAs are also entitled to compare entries in CRFs with corresponding source data and to inform the investigator of any errors or omissions. CRAs will also control adherence to the protocol at the investigator site. They will arrange for the supply of investigational product and ensure appropriate storage conditions are maintained. The CRA will make written reports to the sponsor on each occasion when contact with the investigator is made, regardless of whether it is by e-mail or in person. During monitoring visits, source data verification will be carried out by CRAs and all entries in the CRFs will be compared with the original source documents. The investigator must agree to meet with the CRA at regular intervals

and to cooperate in resolving any queries or findings made during the monitoring process.

Each study site will be appointed two CRAs. The one will be blinded to the study drug and will monitor the quality of data entry by the blinded site investigators. The other will be un-blinded and will monitor the two un-blinded study pharmacists. The visits of the CRAs to each of the study sites will be as follows:

Site initiation visit (SIV) SIV will be done virtually through teleconference using the safe e-Platform of HISS. Before this visit, each site will be shipped with all necessary materials including Investigator Site File, study materials (e.g. lab kits, study specific equipment|), eCRFs, Pharmacy File and initiation presentation handouts. CRAs are responsible to report any changes at the site since the initial evaluation report and confirm site still fulfills all prerequisites established in order to participate to the study. Timeframe of the study, primary contact person, delegation of responsibilities (including Study Coordinator assignment), must be clarified. Blinded CRA will train the site staff to the protocol procedures and familiarize them with the study file and the study web-portal. Special training will be done for the capturing for TEAEs according to the principles analyzed above (see section for adverse events). Overall, the following aspects related to the Protocol must be reviewed during the meeting and subsequently all study participants complete relevant training respectively for:

- Protocol adherence
- Summary of Product (Investigation Study Drug) Characteristics, Investigator's Brochure content
- Case Report Forms completion. All data entries must be performed by designated personnel and the use of data recording will be in compliance with GCP principles and applicable regulatory requirements
- Informed consent obtaining procedure
- Maintenance of study binder as applicable
- Direct access to source data/documents for monitoring, auditing, Institutional Review Board/Independent Ethics Committee review and regulatory inspections. All study information should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification.
- Procedures/documentation for handling, storing and dispensing investigational product

- Handling and shipping of laboratory supplies
- Maintenance and retention of trial related documents (essential documents and all documents that permit the evaluation of the conduct of a study and the quality of data produced (source documents, CRFs, correspondence, study related documents etc)
- ICH-GCP guidelines, Declaration of Helsinki and other regulatory requirements
- Review of all available resources/ facilities/staff
- Investigator responsibility for training trial staff and sub-investigators
- ICF maintenance, maintenance of all study logs
- Additionally, laboratory personnel must be specifically trained for lab samples analysis handling, analysis procedure and storage, completion of required forms.

The un-blinded CRA will train the delegated site pharmacists to keep current a Drug Dispensing Log containing the following information:

- Initial inventory upon receipt of supplies at the study site
- Identification number of each subject to whom test drug was administered
- Date(s), quantities, vial numbers, lot numbers and calculations for all test drugs administered
- Final inventory (upon completion of the study)

Overall, the following aspects related to the Protocol must be reviewed and subsequently all delegated pharmacists complete relevant training respectively for:

- Protocol adherence
- Summary of Product (Investigation Study Drug) Characteristics , Investigator's Brochure content
- Maintenance of study blind as applicable
- Study Drug Preparation, Receipt and Storage
- Handling and shipping of laboratory supplies
- Maintenance and retention of trial related documents
- ICH-GCP guidelines, Declaration of Helsinki and other regulatory requirements
- Review of all available resources/ facilities/staff
- Investigator responsibility for training trial staff and sub-investigators

- Pharmacy File maintenance, maintenance of all pharmacy logs
- Scope and frequency of monitoring visits for both blinded and unblended CRAs must be also discussed.

First monitoring visit The first monitoring visit will take place after enrolment of the first patient at a study site. The purpose of the first visit by the blinded CRA is to monitor the correctness of actions taken during the screening and study visits in order to capture any deviation from the study protocol. During the same visit the un-blinded CRA monitors the delivery of the study medication to the study site, the inventory at the site, the usage for each subject, and destruction. The inventory should contain:

- The subject identification number to whom the drug is dispensed
- The lot number of the drug dispensed
- The date(s) and the quantity of the drug dispensed to the subject

Both CRAs will feedback written reports describing analytically what they have monitored, the deviations found and the proposed corrective actions.

Next monitoring visits Taking into consideration the amount of clinical information that needs to be entered into each eCRF, it is considered that two remote CRA visits are needed per five patients and one remote CRA visit per 10 to monitor for the primary endpoint. The purpose of these visits by the blinded CRA is to monitor the corrective actions suggested at the previous visit and to monitor the correct entry of data from the patient file to the eCRF, the correct storage and processing of blood samples and their transportation for analysis. During the same visit the un-blinded CRA monitors the delivery of the study medication to the study site, the inventory at the site, the usage for each subject, and destruction. In details during monitoring visits following aspects are reviewed;

- Recruitment rate
- Any unresolved issues ongoing since last visit
- All safety issues (adverse event reporting, see section below)
- Consent forms
- Protocol and amendment (if applicable) compliance
- eCRF corrections
- Subject data verification as agreed / possible discrepancies capture

- Subject logs completion
- Study personnel assignment/ site monitoring log update/ new staff training (if applicable)/ISF up-to-date completion
- IMP storage, handling
- Pharmacy logs completion
- Study blind maintenance
- Site facilities

Close-out visit This visit is taking place at the completion of the participation of one study site at the SAVE-MORE study. The purpose of this visit for the blinded CRA is to store all study files and to collect all remaining study material. The purpose of this visit for the blinded CRA is to destroy all drug supplies and to store inventory files. It is strictly stated that the principal investigator or his representative will confirm by signing the conduct of the monitoring visits. Overall, the following aspects must be reviewed during close out visit:

- CRF corrections/queries resolved
- Identifications of subject notes for site retention
- Study drug management, accountability, record and destruction logs
- Possible ongoing safety issues resolution
- Investigator Site File update
- IEC/regulatory end of study notification
- Original Site Monitoring Log/Screening/ Enrolment Log and Delegation Log collection
- Resolution of any ongoing issues

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APPENDIX I List of study sites in Greece

- Department of COVID-19, Evangelismos General Hospital (PI: Ioannis Kalomenidis, Professor of Pulmonary Medicine)
- 4th Department of Internal Medicine, ATTIKON University General Hospital, (PI: Anastasia Antoniadou, Professor of Internal Medicine and Infectious Diseases)
- 1st University Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases of Athens (PI: Professor Antonia Koutsoukou)
- 2nd University Department of Internal Medicine, IPPOKRATEION General Hospital of Athens (PI: Helen Sambatakou, Associate Professor of Internal Medicine and Infectious Diseases)
- 3rd University Department of Internal Medicine, General Hospital of Chest Diseases of Athens I SOTIRIA, (PI: Garyfallia Poulakou, Assistant Professor of Internal Medicine)
- Department of Clinical Therapeutics, ALEXANDRA General Hospital of Athens, (PI: Evangelos Kostis, Director of NHS)
- 1st University Department of Internal Medicine, General Hospital of Athens LAIKO, (PI: Michael Samarkos, Associate Professor of Internal Medicine and Infectious Diseases)
- 1st Department of Internal Medicine, General Hospital of Athens G. GENNIMATAS, (PI: Georgios Adamis, Director of NHS)
- Department of Internal Medicine, General Hospital of Chest Diseases of Athens I SOTIRIA (PI: Aikaterini Argyraki, Senior Registar)
- COVID-19 Department, General Hospital of Attica SISMANOGLEIO-AMALIA FLEMING, (PI: Malvina Lada, Director of NHS)
- 1st Department of Internal Medicine, General Hospital of Athens KORGIALENIO-BENAKIO E.E.S. (PI: Vasiliki Tzavara, Director of NHS)
- 3rd Department of Internal Medicine, General Hospital of Athens KORGIALENEIO-BENAKEIO E.E.S. (PI: Maria Chini, Director of NHS)
- 2nd Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases of Athens (PI: Aggeliki Rapti, Director of NHS)

- 4th Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases of Athens (PI: George Tsoukalas, Director of NHS)
- 5th Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases of Athens (PI: Aikaterini Dimakou, Director of NHS)
- 10th Department of Pulmonary Medicine, SOTIRIA General Hospital of Chest Diseases of Athens (PI: Ilias Kainis, Director of NHS)
- 1st Department of Internal Medicine, General Hospital of Nea Ionia CONSTANTOPOULIO-PATISION (PI: Aikaterini Masgala-Seferli, Director of NHS)
- Department of Internal Medicine, General Hospital of Athens ELPIS (PI: Archontoula Fragkou, Director of NHS)
- 1st Department of Internal Medicine, General Hospital of Eleusis THRIASIO (PI: Styliani Symbardi, Director of NHS)
- 2nd Department of Internal Medicine, General Hospital of Eleusis THRIASIO (PI: Zoi Alexiou, Director of NHS)
- 1st Department of Internal Medicine, General Hospital of Voula ASKLEPIEIO (PI: Ioannis Bliziotis, Registrar of NHS)
- 2nd Department of Internal Medicine, General Hospital of Piraeus TZANEIO (PI: Georgios Chrysos, Director of NHS)
- 1st Department of Internal Medicine, AMALIA FLEMING Prefecture General Hospital of Melissia (PI: Georgios Marathonitis, Director of NHS)
- 1st Department of Internal Medicine, AHEPA University General Hospital of Thessaloniki (PI: Simeon Metallidis, Associate Professor of Internal Medicine and Infectious Diseases)
- 2nd Department of Propedeutic Medicine, Ippokrateion University General Hospital of Thessaloniki (PI: Michalis Doumas, Professor of Internal Medicine)
- 1st Department of Internal Medicine, PAPAGEORGIOU General Hospital of Thessaloniki (PI: Glykeria Tzatzagou, Director of NHS)
- 3rd University Department of Internal Medicine, PAPAGEORGIOU General Hospital of Thessaloniki (PI: Vasileios Kotsis, Professor of Internal Medicine)
- Department of Internal Medicine, University General Hospital of Patras PANAGIA I VOITHIA, (PI: Karolina Akinosoglou, Assistant Professor of Internal Medicine)

- Department of Internal Medicine, University General Hospital of Larissa, (PI: George Dalekos, Professor of Internal Medicine)
- 2nd Department of Internal Medicine, University General Hospital of Alexandroupolis, (PI: Periklis Panagopoulos, Assistant Professor of Internal Medicine)
- 1st Department of Internal Medicine, General University Hospital of Ioannina, (PI: Haralampos Milionis, Professor of Internal Medicine)
- Department of Pulmonary Medicine, General Hospital of Kerkyra (PI: Ilias Papanikolaou, Director of NHS)

Monitor Dr. Antigoni Kotsaki, MD, PhD
e-mail: antigonebut@yahoo.com tel: +30 694 66 37 164; +30 210 58 31 2562

Pharmacovigilance supervisor Ms Areti Voulomenou, MEng, MSc
e-mail: voulomenou@suschem.gr tel +30 210 82 52 510

APPENDIX II

MINUTES OF MEETING HELD ON NOVEMBER 30TH 2020

Chairman of the ETF: Marco Cavaleri

Participants: Radu Botgros, Maria Fernandez, Sara Galluzzo, Evangelos J. Giamarellos-Bourboulis, Regine Lehnert, Eric Pelfrene, Katarzyna Turobos, Richard Vesely

Topic: Advise request for suPAR-guided Anakinra treatment to prevent Severe Respiratory Failure in COVID-19

The applicant, E. J. Giamarellos-Bourboulis thanked the ETF panel for inviting him to present his current work on the personalized treatment of COVID-19.
The Applicant presented slides that were shared with the panel by e-mail prior to the TC. The generated pre-clinical data leading to the SAVE trial and the results of the SAVE trial on personalized treatment with anakinra guided by the use of the biomarker suPAR in order to prevent mechanical ventilation were presented.

The following points were discussed after the end of the first part of the presentation:

Question 1 Anakinra programme is selecting patient population based on presence of only one of the DAMPs. How can we be sure that other DAMPs are not better biomarkers for selecting a population that would respond to anakinra treatment?
Applicant: Other DAMPs than IL-1α are considered to be involved in the pathogenesis of COVID-19. Among them are calprotectin, HMGB1 and endotoxins translocating from the gut. We know specifically that the gut is one organ affected in COVID-19. All the DAMPs are acting through TLRs and they stimulate the production of IL-1β from tissue macrophages. Since anakinra blocks both IL-1α and IL-1β, anakinra is the best candidate to fully attenuate the effect of DAMPs. This is also verified in our animal model that is described in Annex I of the briefing document that I shared with you. In this model, wild-type C57Bl6 mice are intravenously challenged

with plasma from patients with severe COVID-19 and from healthy volunteers for three days. Through this challenge, they acquire one compartmentalized organ inflammatory reaction which is closely mimicking human COVID-19. This makes us believe that COVID-19 is driven through circulating DAMPs. Indeed, anakinra manages to fully attenuate this compartmentalized pro-inflammatory response. At the cell level, the pro-inflammatory response of tissue macrophages is attenuated whereas the Th1 response of lymphocytes, which is needed to contain the virus, is increased.

Question 2 a) can suPAR be used routinely; b) the second cohort is coming from a publication that is not focusing on ARDS but on acute kidney injury; and c) how it behaves in crucial illness?

Applicant: a) suPAR can be measured easily at the emergency department using point-of-care devices and a principle similar to the one of troponin measurement when myocardial ischemia is suspected. The suPAR assay is CE-IVD marked and it is also licensed by the FDA. The manufacturing company is Virogates. b) Indeed, the second cohort of 352 patients (published in November 2020 at the JASN) is focusing on the role of suPAR for the progression of COVID-19 into acute kidney injury. In parallel, the same authors have shown strong prognostic role of suPAR for the risk of mechanical ventilation. c) We got one grant from the EU, with the acronym RISKinCOVID, and we have done suPAR measurements in a cohort of 245 patients under mechanical ventilation. All patients had suPAR more than 10 ng/ml.

Question 3: a) did you look for an association between start of symptoms and response to anakinra; b) how do you comment the non-specific nature of suPAR; c) can suPAR be used for other clinical trials; and d) what are your liaisons to SOBI?

Applicant: a) Indeed, we analysed if there is an association between time delay from start of symptoms to start of anakinra and final outcome and also between time delay from hospital admission to start of anakinra and final outcome. We did not find a direct association. However, subgroup analysis among patients with suPAR ≥6 ng/ml and pO_2FiO_2 >300 mmHg showed that the incidence of SRF was 41.4% and 8.2% among patients receiving standard-of-care (SOC) only and SOC plus anakinra respectively. The respective incidence among patients with suPAR ≥6 ng/ml and pO_2FiO_2 ≤300 mmHg was 73.6% and 34.8%. These data show that the sooner in the time course of COVID-19 (when the respiratory ratio is still >300 mmHg) anakinra

start the better the outcome. However, patients with respiratory ratio ≤300 mmHg also have significant benefit. b) Indeed, suPAR is not an index of response to Anakinra. In the SAVE trial, it is used as a biomarker that selects those patients who are more at risk of unfavourable outcome. suPAR is non-specific and may be increased by other conditions. To avoid this confounding, we excluded from the SAVE study patients at stage IV malignancy, end-stage renal disease and severe liver failure that may increase baseline suPAR. We will repeat these exclusion criteria in the SAVE-MORE trial. c) I believe that suPAR may also be used in other trials to indicate patients at high risk for unfavourable outcome. d) SAVE study was funded in each initial step by one Greek naval company, namely Technomar. It was also funded by the EU grant RISKinCOVID. SOBI provided drug for 37 patients and a small grant to cover expenses for reagents. I do not act on behalf of SOBI.

Question 4: suPAR is not a biomarker that can predict the Anakinra results. How do we deal with the fact that we do not know if Anakinra may have benefit in a broader population or other biomarkers might be more predictive?

Applicant: suPAR is not specific to predict that patients will respond to Anakinra. However, suPAR is showing that a process which is driven through alarmins and which ends to ARDS has started. Since this alarmin-driven cascade is inhibited with anakinra, then using suPAR as a prognosticator or unfavourable outcome is sufficient. Our data, that are described in Annex III of the briefing document, indicate that Anakinra acts at the monocyte level by restoring the balance between the pro-inflammatory and the anti-inflammatory responses.

Question 5: There is another trial that you are running comparing anakinra to tocilizumab using different biomarkers. Can you comment on this?

Applicant: We have just finished the ESCAPE trial. This is a trial in a completely different patient population than the SAVE trial. Patients enrolled in ESCAPE are critically ill whereas patients enrolled in the SAVE trial are not in a state of respiratory failure. In the ESCAPE trial, we deliver intravenous treatment with anakinra in patients with macrophage activation syndrome as this is diagnosed by extremely high levels of ferritin. In the same trial, we deliver intravenous treatment with tocilizumab in patients who have flow cytometry findings compatible with the complex immune dysregulation that is described in our *Cell Host and Microbe* publication. I have talked to you for this immune dysregulation at the first part of my presentation. However,

ESCAPE is far different than SAVE. In the ESCAPE trial we want to treat someone who is already admitted in the ICU. In the SAVE trial, we wish to prevent ICU admission in a patient population at high risk.

Applicant presented the second part of the slides focusing on the SAVE-MORE trial for which he was seeking advice.

The following points were discussed after the end of the second part of the presentation:

Question 6 Personal views are expressed in this TC. In principle your suggested primary outcome measure can be agreeable with the suggestion to measure efficacy at a later timepoint, e.g. 28 days. However, consideration should be given to the use of the 11-point WHO scale that has been published in *Lancet Infectious Diseases.*

Applicant agreed and I would revise the protocol accordingly.

Question 7 Can you better explain your standard-of-care (SOC) therapy as we had the impression that all patients will be treated in the same way although they will not be of the same degree of severity?

Applicant: Dexamethasone will be administered in patients in need for oxygen according to the NIH algorithm. Remdesivir can be used only in selected cases at the discretion of the treating physicians, but not all patients will receive remdesivir in the SOC.

Question 8 a) you need to define criteria for hospitalization since hospitalization is one of your inclusion criteria; and b) you need to explain to us why you selected pO_2/FiO_2 less than 150 mmHg as one of the exclusion criteria.

Applicant: a) Criteria for hospitalization will be clearly stated on the revised protocol, as suggested; and b) $pO_2/FiO_2 < 150$mmHg is set as one of the exclusion criteria since this pO_2/FiO_2 cut-off is part of the primary endpoint. However, we suggest such low pO_2/FiO_2, and not higher, just because our feedback from our investigators is that Anakinra provides benefit even when pO_2/FiO_2 is low and the patient is not in need of mechanical ventilation.

Question 9: There are two other trials, the REMAP-CAP and one in Italy using anakinra. Can you comment on this?

Applicant: Both trials aim to treat patients who are already admitted in the ICU. In the SAVE trial, we wish to prevent ICU admission in a patient population at high risk.

Question 10: Why don't you randomize patients according to the suPAR levels, i.e. including both below and above the threshold defined?

Applicant: As mentioned during the first part of the presentation, 211 patients failed screening because they had suPAR lees than 6 ng/ml; only seven were intubated. This means that the probability for unfavourable outcome will be 3% for placebo treated patients with low suPAR. This will lead to a trial powered for lot of patients for which the clinical meaning will probably be doubtful. This is something that we want to avoid.

Question 11 You do understand that with this approach, if anakinra is approved, the indication of use might be limited to patients with high suPAR?

Applicant: I agree, and I believe that the merit of the SAVE-MORE trial is that we select patients at high risk for unfavourable outcome using suPAR.

Question 12 : a) There are many suggested biomarkers and you need to develop one specific to predict response to Anakinra; b) you need to collect biosamples at more frequent time intervals.

Applicant: We will collect biosamples for transcriptomic and proteomic analysis so as to identify specific biomarkers that predict response to Anakinra. I will revise the protocol to add this as an exploratory endpoint. Protocol revision will also include sampling at one more follow-up time-point i.e. day 4.

Giamarellos-Bourboulis: The SAVE-MORE trial will be run in study sites in Greece, Italy and the Netherlands. This collaboration includes 28 study sites in Greece (all 28 principal investigators have signed the letter addressed to ETF), Prof. G. Ippolito and Prof. M. G. Netea. The biomarker analysis will run in Athens and in Nijmegen. All investigators are committed to provide results and analysis for the primary endpoint in two months. A question was asked pertaining to the situation in which the primary endpoint of SAVE-MORE is successful, whether conditional approval be granted for anakinra for patients with COVID-19 pneumonia and suPAR ≥6 ng/ml. A second question was whether with the results of the SAVE trial conditional approval may already be granted until the results of SAVE-MORE become available.

ETF response: It is unlikely for EMA to provide conditional approval based only on the results of a non-randomized trial. However, if the SAVE-MORE trial is successful in the primary endpoint, ETF may be in the position to advise CHMP to grant conditional approval for patients with COVID-19 pneumonia and suPAR ≥6 ng/ml, following appropriate regulatory submission and evaluation procedure.
The applicant's request and summary of this informal discussion will be shared with Emergency Task Force on Friday 4th December and ETF recommendations to the proposed protocol for SAVE-MORE study will be sent to Applicant with no delay.
ETF is not in a position to grant any kind of marketing authorisation, unauthorised use of medicines or clinical trial approval as these are not in their remit. The document will contain advice how to proceed in this matter.

EMA thanked on behalf of the panel E. J. Giamarellos-Bourboulis for his efforts and for his initiative. E. J. Giamarellos-Bourboulis thanked Dr. Cavaleri and the entire panel for honouring him with this hearing and suggested that he would distribute the minutes from his side by December 1st, 2020 to the Panel. The Panel will edit the minutes and send back to E. J. Giamarellos-Bourboulis by Friday November 4th, 2020 so that he can initiate his actions for the SAVE-MORE trial.

3 December 2020
EMA/659928/2020

Clinical Studies and Manufacturing Task Force (TCS)

EMA Comments on SAVE-MORE Protocol

(suPAR-GUIDED ANAKINRA TREATMENT FOR VALIDATION OF THE RISK AND EARLY MANAGEMENT OF SEVERE RESPIRATORY FAILURE BY COVID-19)

This proposal comes from a group of investigators from Greece, Italy and Netherlands led by Professor Giamarellos-Bourboulis, sponsored by Hellenic Institute for the Study of Sepsis in Athens. It is a multi-centre randomised double-blind placebo-controlled study in hospitalised patients with COVID-19 and suPAR ≥6 ng/mL to compare early anakinra + SOC with SOC + placebo to prevent progression to severe respiratory failure. The study is a follow-up to an ongoing single arm externally controlled SAVE study based on positive results of its interim analysis. As mentioned, the study population is to be selected based on the elevated plasma levels of suPAR (serum urokinase plasminogen activation receptor) that is claimed to be able to identify the population at high risk of progression to severe respiratory failure. Some literature data supporting this claim is to be found in the briefing document and in the presentation provided by investigators.

The study is welcome in view of controversial results of studies using anti-IL-1 treatments and anti-cytokines in general.

If this study were to be considered pivotal or supportive for regulatory decisions for anakinra, there are however some aspects that would deserve consideration for possible amendments or at the very least would benefit from further justifications.

Preliminary comments are summarised below:

Inclusion/exclusion criteria:

The study population is selected based on elevated suPAR (≥6ng/ml). Previous data provided by investigators have described significant relationship with this biomarker and risk of progression to respiratory failure (Rovina, N., Akinosoglou, K., Eugen-Olsen, J. et al. Soluble urokinase plasminogen activator receptor (suPAR) as an early predictor of severe respiratory failure in patients with COVID-19 pneumonia. Crit Care 24, 187 (2020). https://doi.org/10.1186/s13054-020-02897-4). It is acknowledged that urgent validationof suPAR, either as prognostic factor for worse prognosis or as predictive of the response to anakinra and/or other immunomodulators, is not feasible. T The inclusion of a biomarker negative group might significantly reduce the power of the study to detect a sufficient number of patients with disease progression (given that according previous studies only 3% of patients with low levels of suPAR progressed to respiratory failure) the proposed approach is therefore acceptable. It should be however noted that any future regulatory decision on a therapeutic indication would need to reflect the population that was studied.

Stratification should be made based on participating sites or regions.

Exclusion criterion for use of previous biological treatment is accepted and it should be the same for administration of steroids above 0.4 mg/kg.

Clarification on whether it is expected that patients with pO2/FiO2<150 mm would be on NIV should be provided.

For subjects without decision-making capacity, informed consent must be obtained from a legally designated representative following the national legislation in the Member State where the trial is planned.

Concomitant therapy:

Use of steroids, remdesivir or other treatments included in SOC should be more precisely defined. Stratification by site or region as proposed above should be applied.

Interventions:

See comments on Standard of Care above. The study is planned to be conducted in relatively short time (2 months) so it should be possible to define precise SOC for all participating centres

Primary endpoint:

The primary endpoint of respiratory failure defined as progression into hypoxemia with pO_2/FiO_2 <150 mmHg necessitating MV or NIV or ECMO seems reasonable. However, it should be clarified if the physiological parameter is sufficiently sensitive, no matter what medical procedure it instigates, if e.g. PEEP can influence the proposed p/F value and how it is measured.

Use of 11-point of WHO ordinal scale would allow easier comparability with other studies and can be considered. It is recommended that time span for measurement of primary endpoint is extended to 28 days.

Secondary endpoints:

It is recommended to consider adding the following:

- Relation of endpoints to duration of disease (from first symptoms) and timing of treatment initiation
- Analysis of other biomarkers incl. CRP, D-dimer, ferritin, IL-6, in plasma, trascriptomic and proteomic analyses
- Outcome analysis of patients with suPAR<6 ng/ml if included
- Long-term safety follow-up

To understand the dynamics of the disease progression and biomarker dynamics it is recommended to add additional measurement at day 4 to viral load and biomarkers (Day1, 4 and 7).

Statistical considerations:

It would be expected that relevant baseline covariates to be included in the model are pre-specified, and a stepwise approach to their inclusion is generally not supported. Any variables used

to stratify the randomisation should automatically be included, and any other ones strongly predicative or prognostic should also be pre-specified and included. Please see the EMA Guideline on adjustment for baseline covariates in clinical trials (EMA/CHMP/295050/2013) for further explanation of the regulatory position, in particular Section 6.

It is unclear why time-to-event analyses will be considered for adjusted analyses, and interpretation of the rates at D14 may be more clinically interpretable and relevant. Logistic regression or the stratified Cochrane-Mantel-Haenszel test should be considered instead.

The definition of the per protocol set is a little non-standard, although would be suitable for regulatory decision making, as would the ITT set. It may be appropriate to consider and pre-specify how any missing data will be handled in the analysis. Further guidance is available in the EMA Guideline on Missing Data in Confirmatory Clinical Trials (EMA/CPMP/EWP/1776/99 Rev. 1) and the ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials

CLINICAL TRIAL APPLICATION

Before the trial starts, an application must be submitted to and authorised by national competent authorities and ethics committees in each Member State where the trial is planned.

DISCLAIMER:

These comments adopted by EMA Emergency Task Force are not to be considered as acceptance or approval of the clinical study, which is not in the remit of EMA. It is also not to be considered as endorsement of off-label or unauthorised use of any medicinal product.

APPENDIX III Study visits

Visits days	**Screening**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**14**	**28**	**60**	**90**
Informed consent	x	x													
Exclusion criteria	x														
Inclusion criteria	x														
suPAR point-of-care measurement	x														
Extent of radiological lung involvement	x														
Co-morbidities	x														
SOFA score		x	x	x	x	x	x	x	x	x	x	x			
Co-administered drugs		x	x	x	x	x	x	x	x	x	x	x			
Blood sampling for safety	x	x[a]			x			x							
Blood sampling for suPAR, CRP, IL-6, ferritin, D-dimers, transcriptomic and proteomic analysis	x	x[a]			x			x							
11-point WHO Clinical Progression Scale			x	x	x	x	x	x	x	x	x	x			
AE/SAE			x	x	x	x	x	x	x	x	x	x	x	x	x
Study drug administration		x	x	x	x	x	x	x	x	x	x				

[a]not needed if time interval from screening blood sampling is less than 24 hours

APPENDIX IV The SOFA score[12]

Variable	**0 points**	**1 point**	**2 points**	**3 points**	**4 points**
PaO_2/FiO_2(mmHg)	≥400	<400	<300	<200	<100
Platelets (per mm^3)	≥150	<150	<100	<50	<20
Hypotension	MAP≥ 70 mmHg	MAP<70 mmHg	Dobutamine whatever dose	Adrenaline ≤0.1* or Noradrenaline ≤0.1*	Adrenaline >0.1* or Noradrenaline >0.1*
Glasgow Coma Scale	15	13-14	10-12	6-9	<6
Bilirubin (mg/dl)	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
Creatinine (mg/dl) or Urine output	<1.2	1.2-1.9	2.0-3.4	35-4.9 or <500ml/day	≥5.0 or <200ml/day

*µg/kg/min

Each variable is scored between 0 and 4. The SOFA score is the sum of the score of each variable.

APPENDIX V Evaluation of the extent of lung involvement by chest computed tomography (adapted from reference 16)

Please indicate for each lung lobe the appropriate score. Then sum the score of each lobe.

Lung lobe	**Score**	**Interpretation**
Right upper lung lobe	0	No lesion
	1	Less than 1/3 of volume involved
	2	Between 1/3 and 2/3 of volume involved
	3	More than 2/3 of volume involved
Right middle lung lobe	0	No lesion
	1	Less than 1/3 of volume involved
	2	Between 1/3 and 2/3 of volume involved
	3	More than 2/3 of volume involved
Right lower lung lobe	0	No lesion
	1	Less than 1/3 of volume involved
	2	Between 1/3 and 2/3 of volume involved
	3	More than 2/3 of volume involved
Left upper lung lobe	0	No lesion
	1	Less than 1/3 of volume involved
	2	Between 1/3 and 2/3 of volume involved
	3	More than 2/3 of volume involved
Left lower lung lobe	0	No lesion
	1	Less than 1/3 of volume involved
	2	Between 1/3 and 2/3 of volume involved
	3	More than 2/3 of volume involved

APPENDIX VI The WHO Clinical Progression Scale (adapted from reference 12)

One score of this scale needs to be assigned by the investigators according to the study visits (see also Appendix III)

Patient state	**Descriptor**	**Score**
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalized; moderate disease	Hospitalized; no oxygen needed	4
	Hospitalized; oxygen by mask or nasal prongs	5
Hospitalized; severe disease	Hospitalized; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation $pO_2/FiO_2 \geq 150$ or $SpO_2/FiO_2 \geq 200$	7
	Mechanical ventilation $pO_2/FiO_2 < 150$ ($SpO_2/FiO_2 < 200$) or vasopressors	8
	Mechanical ventilation $pO_2/FiO_2 < 150$ and vasopressors, dialysis or ECMO	9
Dead	Dead	10